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Smoking Is Associated with Alterations of Blood Thiol-Group Related Antioxidants in Patients on Hemodialysis

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Key Words
Glutathione · Smoking · Hemodialysis · Lipid peroxides

Abstract
Cardiovascular disease is the major complication and cause of mortality in the dialysis population, accounting for about 40% of deaths. Oxidative stress has been strongly implicated in the pathogenesis of these events. As patients in end-stage renal disease (ESRD) are in a state of elevated free radical activity, the aim of the present study was to investigate the negative impact of smoking in 45 male hemodialysis (HD) patients. These patients, who were 40–85 years of age (mean age 60.9 ± 13.3 years), had been on hemodialysis for at least 12 months before participating in this study. Fasting blood sampling for serum lipid, albumin, urate, lipid peroxides total blood glutathione (tGSH), non-GSH free sulfhydryl compounds (non-GSH fSH), plasma glutathione peroxidase (pGSHPx), erythrocytes glutathione peroxidase (rGSHPx), plasma glutathione S-transferase (pGST) and erythrocytes glutathione S-transferase (rGST) were determined. Our study showed that the plasma malonyldialdehyde (MDA) concentration was significantly higher in HD patients who smoked than in those who were non-smokers (1.99 ± 0.53 vs. 1.55 ± 0.46 nmol/ml, p = 0.008). No association was found between levels of MDA in smokers and BMI, serum cholesterol and triglycerides and smoking index. We also found that the circulating plasma levels of tGSH and non-GSH fSH was lower in the HD patients who smoked (tGSH 164.9 ± 41.5 vs. 203.4 ± 45.3 μg/ml; fSH 271.1 ± 55.8 vs. 308.8 ± 46.7 μg/ml; $p < 0.05$ and $p < 0.001$, respectively). There were no significant differences in the plasma levels of uric acid, pGSHPx, rGSHPx, pGST, rGST, albumin, and age between the 2 groups. Partial correlation analysis of the plasma levels of the measured antioxidants and the smoking index revealed a negative correlation between the plasma levels of tGSH and smoking index ($r = -0.62$, $p < 0.003$). Similarly, the plasma levels of tGSH was found negatively correlated with the levels of plasma MDA ($r = -0.32$, $p < 0.05$) of the HD patients. In conclusion, our data suggest that cigarette smoking has a negative impact on plasma-circulating products of lipid peroxidation in HD patients. The lower blood levels of the tGSH and non-GSH fSH in HD patients who smoked suggests that these patients may be more susceptible to oxidative damage caused by smoking.

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Introduction

Cardiovascular mortality is substantially elevated in dialysis patients and the risk for cardiovascular mortality is approximately 10 times (range 2.5–17.0) times higher than that of the general population [1, 2]. Many dialysis- and uremia-related factors can contribute to the genesis of atherosclerotic disease [3]. Attention has recently been directed toward the potential role of oxidative stress in uremic cardiovascular complications.

Oxidative stress, defined as a perturbation in the pro-oxidant and antioxidant balance, has been strongly implicated in both atherosclerosis and generalized myocardial dysfunction in diverse human and animal studies [4]. End-stage renal disease is characterized by an increased level of the free radical oxidants relative to the antioxidants. Several previous studies [5–6] have demonstrated that ESRD patients on maintenance hemodialysis were found to be exposed to higher oxidative stress of free radicals and reactive oxygen species (ROS). Some investigators [7–9] have shown increased lipid peroxidation in the plasma and erythrocytes of uremic patients [7–9]. Probably an imbalance between the generation and the removal of ROS and free radicals associated with the uremic milieu play a pivotal role in the atherosclerotic complications in patients with ESRD.

Cigarette smoking has been firmly established as one of the major cardiovascular risk factors in non-ESRD populations. Some studies investigating patients on hemodialysis document that smoking substantially increases the risk of death from cardiovascular complications, particularly in patients with diabetes mellitus [10]. Cigarette smoke contains a wide variety of chemically reactive species that may lead to the modification of biological macromolecules [11]. Oxidative stress is considered to be one of the major pathogenetic mechanism associated with smoking [12]. It has been hypothesized that the active chemical substances damage lipids and result in the formation of pro-atherogenic oxidized particles, specifically oxidized LDL [13]. Several studies have demonstrated higher levels of oxidized LDL in smokers [13, 14]. Moreover, cigarette smoke has been reported to result in depletion of antioxidant vitamins in the body [15–16]. All these pathologic effects may probably provide an important causal mechanism that links smoking with the development of atherosclerosis in cigarette smokers. In some recent studies, increased susceptibility of oxidative DNA damage has been demonstrated in nonuremic smokers as compared with nonsmokers [17–18].

Glutathione (GSH) is usually the most abundant cellular thiol. It is involved in the transport of certain amino acids, a coenzyme for various enzymes and more importantly, and defends the tissue cells against damage induced by ROS and free radicals. In addition, it also protects cells from toxic effects of xenobiotics and environmental pollutants by serving as a substrate in the removal of metabolic intermediates such as hydrogen peroxide, organic hydroperoxides and lipid peroxides by GSH peroxidase. Alterations of these antioxidants may thus render the body more vulnerable to oxygen free radicals. Most of the smoking-mediated free radicals and ROS may cause alterations of the levels of GSH and related antioxidants [19]. These changes may lead to oxidative damage of cells and tissues. To our knowledge, available data regarding the impact of cigarette smoking on alterations of GSH and related antioxidants and lipid peroxidation in ESRD patients on hemodialysis (HD) are limited. We undertook this study to assess the detrimental effects of cigarette smoking on the plasma levels of thiols related antioxidants in ESRD patients on hemodialysis.

Patients and Methods

The study group consisted of 45 ESRD patients on hemodialysis at the Dialysis Center, Kuang Tien General Hospital. These patients, who were 40–85 years of age (mean age 60.9 ± 13.3 years), had been on hemodialysis for at least 12 months before participating in this study. Patients with neoplasia, acute infectious diseases, severe malnutrition (serum albumin below 2.8 g/dl), severe intercurrent illness, and advanced liver diseases were excluded from this study. The cause of ESRD in studied patients were chronic glomerulonephritis in 32 patients (71.3%), diabetic nephropathy in 4 (8.8%), chronic pyelonephritis in 6 (13.3%), polycystic kidney disease in 1 (2.2%), and with unknown cause in 2 patients (4.4%). All the patients received 4-hour bicarbonate hemodialysis treatment three times a week to maintain a minimum Kt/V urea index of 1.4 per session. Hemodialysis was performed with a hemophan membrane (Gambro Alwall Plus-15 and Plus-18; 1.5–1.8 m^2 surface area) and heparin was used for anticoagulation for all patients.

A control group was established from 20 healthy non-smoking volunteers (recruited from health checkup) who were age-, and sex-matched to the group of ESRD patients. All participants underwent a medical history and physical examination. None of the controls suffered from acute infectious disease, chronic inflammatory diseases or had clinical evidence of any cardiovascular disease. Information on smoking habits was assessed in all patients using a questionnaire. Smoking habits were categorized into those who had never smoked and current smokers. The smoking index was defined as the number of cigarettes smoked per day × years of smoking/20. 3-Day food records were analyzed to determine the vitamin C and A content of the patients' diet [20]. None of our patients were on hypolipidemic drugs and each patient in our study received a daily vitamin B complex supplement.

Collection and Preparation of Blood Samples

After 12 h of fasting, venous blood samples were taken from each of the patients and control subjects. Following centrifugation at 2,000 rpm for 10 min, the plasma was collected and stored in aliquots at –80°C or used immediately for enzymatic assays. Several aliquots of the same samples were transferred into other tubes to be used for the assays of other plasma or serum parameters, which were performed by routine laboratory techniques. In addition, an aliquot of 1 ml fresh blood was collected in a heparin vacutainer for biochemical analysis of the plasma level of lipid peroxides.

Determination of Total Glutathione (tGSH)

An aliquot of 0.05 ml of 10% perchloric acid (PCA) was added to 0.1 ml of whole blood to remove proteins by precipitation and centrifugation. Total free GSH in whole blood was measured with the recycling enzymatic assay, which employs glutathione reductase to induce a kinetic colorimetric reaction of DTNB (5,5'-dithiobis-(2-nitrobenzoic acid) [21]. The rate of change of absorbance at 412 nm was monitored at 30°C within 5 min. The concentration of tGSH in blood was calculated from a standard curve and is expressed as μg/ml.

Determination of Non-GSH Free Sulfhydryl in Whole Blood

The concentration of blood thiols was determined by a colorimetric method according to Ellman [22]. An aliquot of 0.05 ml 10% PCA was added into 0.1 ml whole blood to remove proteins by precipitation and centrifugation. The supernatant was added with 0.05 ml of 0.1 *M* DTNB. After thorough mixing, the mixture was left standing at room temperature. The final absorbance at 412 nm was recorded after 30 min reaction of DTNB with all the SH group-containing compounds. The blood concentration of total free thiols is expressed as μg/ml.

Measurement of GSHPx Activities in Plasma and Erythrocytes

Glutathione Peroxidase (GSHPx). Plasma or hemolyzed erythrocytes, GSH reductase and GSH were incubated in 1.0 ml of 50 m*M* phosphate buffer (pH 7.0) at 37°C for 10 min. NADPH solution (1.0 m*M*) and 5 m*M* sodium azide were then added and allowed to equilibrate for 3 min at 20°C. The enzymatic reaction was initiated by addition of 5 m*M* t-butyl-hydroperoxide as the substrate. The conversion of NADPH to $NADP^+$ was then followed by continuous recording of the decrease of the absorbance at 340 nm for 5 min [23]. GSHPx activity is expressed as U/l or U/g Hb.

Glutathione S-Transferase in Plasma and Erythrocytes (pGST and rGST). The GST activity in the plasma or erythrocytes was measured using 1-chloro-2,4-dinitrobenzene (CDNB) as substrate according to the method described by Habig et al. [24]. The reaction mixture in 2 ml contained 0.2 *M* sodium phosphate buffer (pH 6.5), 0.95 m*M* CDNB, 0.95 m*M* GSH, and 0.5 ml plasma or lysed erythrocyte suspension. The reaction was initiated by the addition of the electrophilic substrate CDNB and the reaction was monitored at 25°C spectrophotometrically by the increase of the absorbance at 340 nm. The background rate in the absence of test samples was subtracted to correct for the nonenzymatic reaction. The GST activity is expressed as U/l or U/g Hb.

Measurement of Plasma Lipid Peroxides

Plasma level of lipid peroxides was measured as malondialdehyde (MDA) by an HPLC system as described previously [25]. 500 μl each of a plasma sample, reagent blank (distilled water), and tetramethoxy-propane (TMP) working standard solutions were respectively pipetted into a glass tube. After mixing, 750 μl of 0.44 *M* phosphoric acid and 250 μl of 0.6% thiobarbituric acid (TBA) were added. The mixture was heated for 1 h in boiling water (100°C), and then cooled at 4°C. This was followed by neutralizing 500 μl of the boiled sample with 500 μl of methanol-1 *N* NaOH mixture (45.5:4.5, v/v) to precipitate proteins before injection into a C_{18} column. After centrifugation, 10–20 μl of the protein-free supernatant was injected to the HPLC system to separate the MDA-TBA adduct from interfering chromogens. The MDA-TBA adduct was quantified spectrofluorimetrically at 532 nm. The concentration of lipid peroxides in the plasma sample is expressed as nmol MDA/ml. A calibration curve was prepared for each run by using 1,1,3,3-tetramethoxypropane as the standard.

Laboratory Evaluation

Biochemical assays of serum albumin, blood urea nitrogen [BUN], creatinine, and uric acid were performed on a multiparametric autoanalyzer (Dimension®, Dade Behring Inc., Newark, N.J., USA). C-reactive protein was determined using a nephelometry method (Behring, Marburg, Germany). Serum ferritin concentration was determined by a microparticle enzyme immunoassay (IMX, Abbott, Chicago, Ill., USA). Serum cholesterol, HDL-cholesterol and triglycerides were determined by commercially available enzymatic kits. Serum LDL-cholesterol was calculated according to the Friedewald formula.

Statistical Analysis

Data are presented as means ± SD. Data analyses were performed with SPSS software. Statistical evaluations were made using Mann-Whitney test for unpaired data and one-way analyses of variance (ANOVA) with Scheffé's test for multiple comparison among means were used to compare intergroups differences. Spearman's correlation coefficient was used for correlation comparison. $p < 0.05$ was considered to be statistically significant.

Results

The main clinical features and biochemical characteristics of 45 male ESRD patients and 20 control subjects who participated in the study are given in table 1. As shown in table 1, HD patients had significantly higher plasma MDA levels as compared with age-matched healthy nonsmoking controls. The average GSHPx activity in plasma and erythrocytes of the dialysis patients was significantly lower than controls. The erythrocyte GST activity in healthy controls and HD patients is also shown in table 1. Healthy controls displayed a mean control value of 5.74 ±0.30 U/g Hb. In the ESRD patients on HD, GST activity of erythrocytes increased significantly with respect to the control values ($p < 0.001$). In contrast, the plasma activity of GST was significantly decreased in the HD patients. In addition, both the mean concentration of total blood GSH (tGSH) and non-GSH free sulfhydryl

Table 1. Main clinical characteristics of HD patients and healthy control subjects

	Control subjects	HD Patients
n	20	45
Age, years	57.7±13.3	60.9±13.3
BMI, kg/m²	24.1±1.72	22.2±3.28
Cholesterol, mg/dl	161.3±32.4	177.2±39.8
Triglyceride, mg/dl	130.8±80.6	143.6±88.6
Total plasma MDA, nmol/ml	0.89±0.26	1.75±0.53[a]
Plasma fSH, μg/ml	338.8±11.1	292.1±53.8[a]
Plasma tGSH, μg/ml	201.0±10.0	186.3±47.3[b]
pGSHPx, U/ml	636.6±77.4	288.6±61.8[a]
rGSHPx, U/g Hb	62.4±2.0	53.8±9.8[a]
pGST, U/l	7.5±0.2	4.8±1.4[a]
rGST, U/g Hb	5.7±0.3	7.8±2.6[a]

Values expressed as mean SD.

Body mass index (BMI), malondialdehyde (MDA), total blood glutathione (tGSH), non-GSH free sulfhydryl compounds (non-GSH fSH), plasma glutathione peroxidase (pGSHPx), erythrocytes glutathione peroxidase (rGSHPx), plasma glutathione S-transferase (pGST) and erythrocytes glutathione S-transferase (rGST).

Comparison by Mann-Whitney test.

[a] $p < 0.001$; [b] $p < 0.05$.

Table 2. Clinical and biochemical characteristics of HD patients grouped according to smoking habits

	Nonsmokers (n = 25)	Smokers (n = 20)
Age, years	60.7±13.6	61.1±13.1
BMI, kg/m²	21.1±3.4	22.4±3.2
Duration of HD, months	30.2±8.2	31.4±5.1
Total cholesterol, mg/dl	179.2±43.5	175.1±35.5
Triglycerides, mg/dl	122.6±56.1	171.2±90.1
HDL cholesterol, mg/dl	38.2±11.6	35.2±12.8
LDL cholesterol, mg/dl	121.1±34.8	116.1±38.4
Fasting blood glucose, mmol/l	15.6±6.7	15.1±5.3
Creatinine, μmol/l	963.6±212.2	1,043.1±309.4
Blood urea nitrogen, mmol/dl	24.6±4.2	22.8±3.9
Serum albumin, g/l	40.6±3.2	41.0±4.9
Urate, μmol/l	440.3±95.2	410.6±113.1
Hemoglobin, g/dl	10.5±0.9	10.4±0.9
Hematocrit, %	31.0±2.8	30.8±2.8
C-reactive protein, mg/dl	0.78±0.72	0.85±1.10
Ferritin, μg/l	575.3±266.5	699.7±496.7
Iron saturation, %	34.4±12.0	44.0±21.1
Systolic BP, mm Hg	136.5±18.1	140.1±20.1
Diastolic BP, mm Hg	82.0±8.7	83.2±10.1

Values expressed as mean SD.

Body mass index (BMI), blood pressure (BP), hemodialysis (HD), high-density lipoprotein (HDL), and low-density lipoprotein (LDL).

Comparison by Mann-Whitney test.

compounds (fGSH) of HD patients were significantly lower than that of the healthy controls.

In table 2, the clinical and biochemical characteristics of HD patients subdivided into groups according to smoking status are reported. The two groups of patients were comparable for all potential confounders. No significance difference was found in age, body mass index (BMI), lipids profile, blood pressure, duration of hemodialysis. Most of the patients in this study were nondiabetics with only 2 in each group described above. There were no differences in dietary intake of vitamin C, and E between smokers and nonsmokers as reflected from their dietary diary. As stated previously, our healthy nonsmokers had significantly lower plasma levels of total MDA than uremic nonsmokers. The plasma MDA concentration was significantly higher in HD patients who smoked than in HD patients who were nonsmokers (1.99 ± 0.53 vs. 1.55 ± 0.46 nmol/ml, $p = 0.008$). We found that the circulating blood levels of tGSH and fSH were lower in the HD patients who smoked (tGSH 164.9 ± 41.5 vs. 203.4 ± 45.3 μg/ml; fSH 271.1 ± 55.8 vs. 308.8 ± 46.7 μg/ml; $p < 0.05$ and $p < 0.001$, respectively) as shown in table 3. No association was found between levels of MDA in smokers and BMI, serum cholesterol and triglycerides and smok-

Table 3. Concentrations of antioxidants and lipid peroxides in the plasma and RBC of HD patients grouped according to smoking habits

	Nonsmokers	Smokers
Plasma fSH, μg/ml	308.8±46.7	271.1±55.8[a]
Plasma tGSH, μg/ml	203.4±45.3	164.9±41.5[a]
pGSHPx, U/ml	273.5±53.2	307.6±67.7
rGSHPx, U/gHb	56.4±8.9	50.6±10.1
pGST, U/l	4.7±1.3	4.9±1.5
rGST, U/gHb	8.2±2.4	7.3±2.7
MDA, nmol/ml	1.55±.46	1.99±0.53[a]

BMI = Body mass index; MDA = malondialdehyde; tGSH = total blood glutathione; non-GSH fSH = non-GSH free sulfhydryl compounds; pGSHPx = plasma glutathione peroxidase; rGSHPx = erythrocytes glutathione peroxidase; pGST = plasma glutathione S-transferase; rGST = erythrocytes glutathione S-transferase.

ANOVA with least significant difference was used to compare the levels of antioxidants of patients in 2 groups and controls.

[a] Significantly different from group 1 (nonsmokers) ($p < 0.05$).

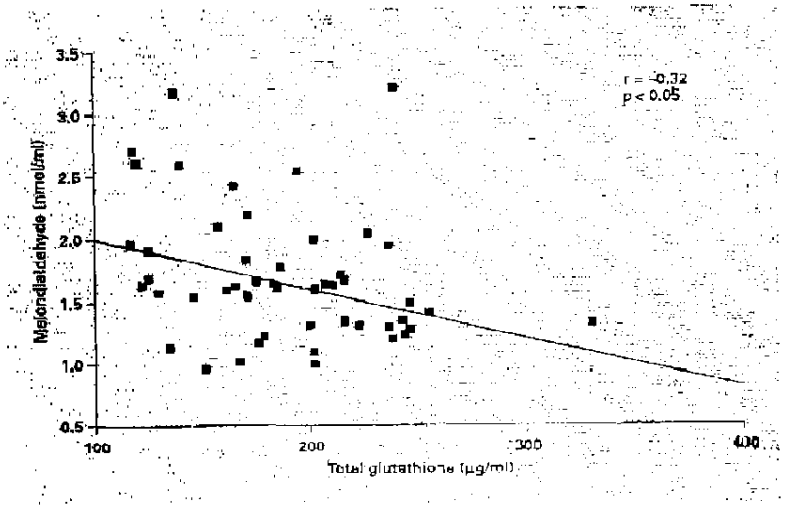

Fig. 1. Relationship between the blood level of total glutathione and that of plasma malondialdehyde for all hemodialysis patients (n = 45). r indicates Spearman correlation coefficient.

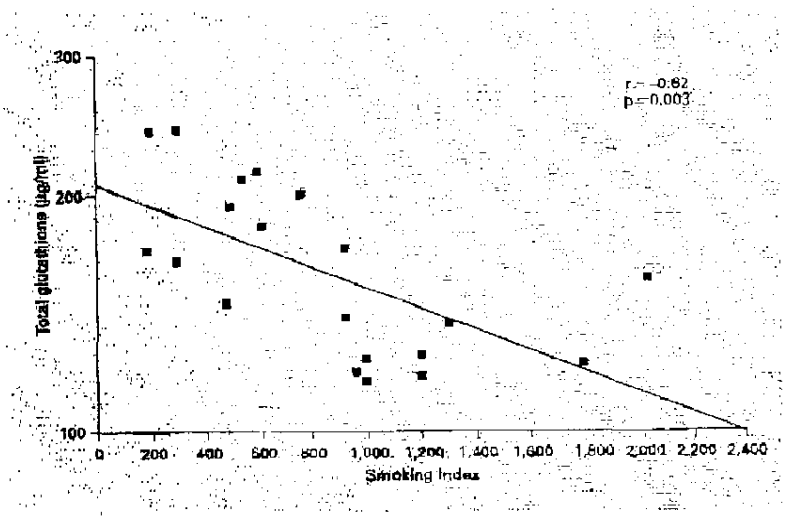

Fig. 2. Regression of the blood level of total glutathione (μg/ml) on smoking index for all hemodialysis patients who smoke (n = 20). r indicates Spearman correlation coefficient.

ing index. As also shown in table 2 and 3, there were no significant differences in the plasma levels of uric acid, pGSHPx, rGSHPx, pGST, rGST, albumin, and age between the 2 groups. Again, neither the systolic blood pressure, diastolic blood pressure, nor glycemic status showed significant difference between the 2 groups.

Apart from the significantly lower levels of plasma glutathione observed in smokers than in nonsmokers on HD, the blood levels of tGSH was negatively correlated with the levels of plasma MDA ($r = -0.32$, $p < 0.05$) (fig. 1). By partial correlation analyses of the measured glutathione-related antioxidants, we found that both blood levels of fSH and plasma levels of rGSHPx were also significantly correlated in a negative manner with the levels of plasma MDA in HD patients (fSH: $r = -0.27$, $p < 0.05$; rGSHPx: $r = -0.30$, $p < 0.05$). In addition, correlation analysis of the plasma levels of the measured antioxidants and the smoking index revealed a negative correlation between the blood levels of tGSH and smoking index ($r = -0.62$, $p < 0.003$) (fig. 2). However, no correlation between the blood levels of fSH or plasma MDA and smoking index was observed. In addition, we found that the blood con-

centration of tGSH exhibited a 7% decrease in both smoking and non-smoking HD patients when compared to controls. On a further analysis, we found that HD patients who smoked had a greater (18%) decrease of tGSH when compared to normal controls.

Discussion

This study demonstrates that smoking caused changes in the indices of thiol-group-related antioxidant status and lipid peroxidation in HD patients. HD patients who were current cigarette smokers have higher measures of lipid peroxidation than nonsmokers as reported previously [26]. Cigarette smoking decreased activity of pGSHPx and lowered blood concentration of tGSH and fSH in HD patients. Alterations of thiol-related antioxidants observed in HD patients who smoke supports the hypothesis that cigarette smoke causes impairment in the enzymatic antioxidant defense and increases oxidative damage of critical biological substances in the body of HD patients.

The thiol-group-related antioxidants, including fGSH, tGSH, GSHPx and GST, constitute one of the major free radical scavenging systems involved in the elimination of the smoking-mediated prooxidants [27]. A decreased efficiency in the natural antioxidant systems presumably exacerbates the extent of the oxidative stress of ESRD patients. Glutathione, a sulfide-containing tripeptide, is present in all cell types and can scavenge H_2O_2, $OH\cdot$, $^1O_2$, and chlorinated oxidants. It also protects cells from the toxic effects of free radicals and other organic hydroperoxides by serving as a substrate for GSHPx and GST during the removal of metabolic intermediates. It is well known that superoxide dismutase and GSHPx acting in tandem provide the primary enzymatic antioxidant defenses [28]. During this process, the level of GSH is believed to be a limiting factor which requires the maintenance of a high GSH/GSSG ratio as achieved by glutathione reductase (GSSG-Rd). Due to its high content of oxidants, the cigarette smoke is bound to cause a prooxidant/antioxidant imbalance in the blood and tissues of smokers. In fact, alterations in antioxidant activities of erythrocytes, tissues and plasma, under smoking-mediated free-radical load, have been observed by several investigators [19, 29, 30]. Toth et al. [29] have found that the erythrocytes from cigarette smokers contain more glutathione and protected endothelial cells against H_2O_2 better than did erythrocytes from nonsmokers. In a separate study, Michelet et al. [29] found that the blood level of tGSH in smokers was positively correlated with the number of cigarettes smoked per day. In the present study, no obvious changes in the blood levels of tGSH and fSH were seen between age-matched normal controls and dialysis patients who did not smoke. However, a decrease in the activity of tGSH and fSH in whole blood has been observed in dialysis patients who smoked. In accordance with our previous work [31], a negative correlation between lipid peroxides and tGSH as well as fSH was observed. Enhanced lipid peroxidation in dialysis patients who smoke may thus be causally related to the antioxidant depletion. In non-ESRD populations, age is an important factor in determining the adaptive response to counteract the smoking-mediated pro-oxidants. With aging, impairment of the regeneration of thiol-group-containing antioxidants was noted [29, 32]. In most of our patients (>40 years old), the thiol-group compounds in the blood circulation are maintained at sufficiently high levels by the adaptive response probably through induction of biosynthesis of GSH from the liver and peripheral tissues. However, in dialysis patients who smoke, the blood concentration of tGSH and fSH is substantially decreased. In fact, we found that the levels of tGSH was negatively correlated with the smoking index ($r = -0.32$; $p < 0.05$).

Since many smoking-mediated mutagens or carcinogens bear electrophilic and/or nucleophilic structure moieties, the activity of GST that catalyzes the conjugation of xenobiotics with GSH, is important in reducing the susceptibility of tissues to the damage caused by these deleterious compounds [24]. Our previous study [33] has shown that the GST activity was increased in the erythrocytes of HD patients when compared to normal controls. However, in the present study, we found that the activity of rGST in the blood of HD patients who smoked was not maximally increased. In a uremic milieu, maximal induction GST could be a response to the low efficiency of the GST-dependent detoxification system which tends to become defective during the aging of uremic erythrocytes [34]. The observed decrease in rGST activity in HD patients who smoked suggests that smoking-mediated oxidants and organic free radicals may be responsible for partial inhibition of this detoxification system.

GSHPx is an antioxidant enzyme which catalyzes the reduction of organic hydroxyperoxides and hydrogen peroxide, using glutathione as the reducing agent, thus protecting cell membranes against lipid peroxidation [35]. In the blood, two types of GSHPx have been identified, namely classical cellular GSHPx in erythrocytes and extracellular GSHPx in plasma. This study has shown that GSHPx activity is decreased in renal failure confirming

the results of previous reports [36, 37]. In contrast, erythrocytes GSHPx activity was not decreased in our HD patients. Since cigarette smoke is potentially capable of generating a high free radical load and many of our male HD patients were smokers, it was prudent to study the causal relationship between long-term smoking and free radical scavenging activity in these patients. In non-ESRD populations, several authors [38–41] have found that the activity of GSHPx is decreased in smokers. It was also found that the GSHPx activity was negatively correlated with tobacco consumption [39]. However, in some studies [31, 40] young smokers exhibited increased activity of GSHPx, whereas decreased activity was seen only in older smokers. Since our patients were comprised of a group of older smokers receiving HD, the process of adaptation seen in younger smokers was not seen. There were also no differences in rGSHPx between HD patients who smoked and who did not smoke.

The role of lipid peroxidation in the pathogenesis of atherosclerotic complications of ESRD patients remains unclear. Cigarette smoke contains a large number of oxidants and can induce lipid peroxidation in plasma and erythrocytes [41] as well as potentially atherogenic changes in lipoproteins [42]. In vitro studies have supported the evidence of an increased formation of lipid peroxides after exposure of plasma to gas phase of cigarette smoke [43], even though the mechanisms by which smoking may increase plasma lipid peroxides remain speculative. The extents of lipid peroxidation in smokers of nonuremic populations have yielded inconsistent results [44–46]. Such reported difference stemmed largely from the limitations of most assays of lipid peroxidation [47] and the different dietary pattern of studied subjects. Although most patients received routine multiple vitamin supplements, the prescription did not include vitamin C and E. Moreover, the HD patients were having diets that were rather restricted in nutritional sources of antioxidants. There were also no apparent differences in surrogate nutritional indices including serum albumin, cholesterol, BMI between smokers and nonsmokers in our study population. These findings suggest that the overall nutritional status of our patients was similar. Our results are in good agreement with published data that the plasma levels of total MDA were significantly higher in HD patients than that of healthy controls [7–9]. Besides, it is more noteworthy to mention that the plasma MDA levels of HD patients who smoked were significantly higher than those of nonsmoking HD patients. Since HD patients had higher baseline MDA concentrations than that of healthy controls, the further increased plasma MDA concentration found is probably explained by cigarette smoking. However, in the present study we did not find any significant relation between plasma MDA levels and number of cigarettes smoked per day.

Limitations of our study design merit consideration as our patients comprised of a group of hemodialysis patients with different underlying renal diseases. In an attempt to ascertain possible interfering influences, we carefully match those patients who smoked and who did not smoke for age, body mass index, duration of hemodialysis and the number of patients with diabetes. In addition, the relatively small number of patients limits the statistical power of the study.

In conclusion, the results from our study suggest that cigarette smoking has a negative impact on plasma-circulating products of lipid peroxidation in HD patients. The finding of lower blood levels of the tGSH, and fSH in HD patients who smoke suggests that HD patients who smoke may be more susceptible to oxidative modification as well as damage elicited by cigarette smoking. Moreover, we found that the tGSH correlated negatively with the amount of lipid peroxides and the smoking index in HD patients. These findings support the clinical importance of discouraging the initiation of smoking as well as of promoting its cessation in HD patients. In this context, it is of interest that smoking cessation not only reduced the cardiovascular risk, but also attenuates the rate of progression of diabetic and nondiabetic renal diseases [10].

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